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(71)出願人　999999999
東洋紡績株式会社
大阪府大阪市北区堂島浜２丁目２番８号

(72)発明者　上村　彰一
滋賀県大津市堅田２丁目１番１号　東洋紡績株式会社総合研究所内

(72)発明者　高杉　浩
滋賀県大津市堅田２丁目１番１号　東洋紡績株式会社総合研究所内

(74)代理人　弁理士　植木　久一　(外１名)

審査官　加藤　浩

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(54)【発明の名称】　細胞培養装置

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【特許請求の範囲】

【請求項１】選択透過性を有する複数の中空繊維内に培養液を加圧下に供給してその一部を該中空繊維の外周側へ透過させ、当該外周域に形成される細胞培養空間において細胞培養を行なう様に構成された細胞培養装置において、

前記中空繊維内に供給された培養液を加圧下に循環させる培養液循環系と、

前記細胞培養空間内で生成した低分子物質及び高分子有用物質を含んだ培養液を、前記低分子物質を分離する為の膜分離装置を介して循環させる生成液循環系を備え、更に、前記培養液循環系と生成液循環系の流量バランスを保持する圧力調整手段を設けたものであることを特徴とする細胞培養装置。

【発明の詳細な説明】

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［産業上の利用分野］

本発明は細胞培養装置に関し、詳細には細胞に効率よく栄養分を与えると共に、産生有用物質を濃縮しつつ副生する不要物質を巧みに除去して系内の環境の均一化を達成し、高密度に連続的細胞培養を行なうのに最適な中空繊維型細胞培養装置に関するものである。

［従来の技術］

高密度な細胞大量培養は、モノクロナール抗体，リンホカインその他の有用な生理活性物質の工業的規模の生産を実現する上で欠かせない技術である。

古典的な細胞培養は培養びん等を用いて実験室レベルで行なわれてきたのであるが、より高密度に且つ大量に細胞培養できれば工業的に有利であり、この様な観点から細胞大量培養の為の方法や装置が各種開発されるに至っている。

これらの方法や装置における培養方式は、培養される細胞の特性によって浮遊性（非付着性）細胞培養と付着性（壁付着性）細胞培養に大別され、前者の浮遊性細胞培養に当たっては細胞の懸濁状態の維持と効率の良い物質交換を、一方付着性細胞培養に当たっては単位容積当たりの付着細胞数の増大及び栄養物，老廃物，気体等の物質交換を効率よく達成することが重要なポイントとなる。

上記いずれの培養方式を採用するにせよ、物質交換が重要なポイントであるところから、選択分離性を有する中空繊維や毛細管を用いた細胞培養が注目され、例えば中空繊維を用いて行なう物質交換は、in vivoで細胞が血管を通じて血液から栄養物を得ると共に代謝産物を排出するのに類似しており、in vitroの細胞培養法として極めて有望視されており、各種の培養装置が開発されるに至っている。中でも中空繊維中に液体培地（培養液）を流して中空繊維外表面に細胞を付着させて増殖させる方法や、中空繊維内に酸素を含む気体を流して中空繊維外表面における液体培地中に存在する細胞のガス交換を積極的に行なう等の各種の改良が進められている。この様な中空繊維を用いた細胞培養技術としては、これまでに特開昭49－41579号，同50－36684号，同52－125688号，同59－175877号，同61－25477号，公表特許昭62－500356号，同62－500357号等の技術が開示されている。

［発明が解決しようとする問題点］

細胞の高密度培養に対する制限因子としては、系内での成育阻害物質（例えば乳酸，アンモニウムイオン等の代謝産物）の蓄積、必須栄養源（例えばグルコース，必須アミノ酸，ビタミン等）の枯渇、溶存酸素量の枯渇、更にはpHの急激な低下等が挙げられる。

従来の中空繊維型細胞培養装置では、この様な制限因子を完全に排除することを目的として開発されていたものであるが、高密度な細胞培養を確立した技術とは言い難い状況であった。特に培養装置内における環境が完全均一状態とはならず、部分的に細胞の生育が不十分となり、その結果培養装置全体としての細胞密度が期待されるほど高くならないというのが実情であった。

本発明はこうした技術的課題を解決する為になされたものであって、その目的とするところは、細胞培養装置内における環境を可及的均一とし、これによって高密度に細胞培養を行なうことのできる中空繊維型細胞培養装置を提供することにある。

［問題点を解決する為の手段］

上記目的を達成し得た本発明とは、選択透過性を有する複数の中空繊維内に培養液を加圧下に供給してその一部を該中空繊維の外周側へ透過させ、当該外周域に形成される細胞培養空間において細胞培養を行なう様に構成された細胞培養装置において、

前記中空繊維内に供給された培養液を加圧下に循環させる培養液循環系と、

前記細胞培養空間内で生成した低分子物質及び高分子有用物質を含んだ培養液を、前記低分子物質を分離する為の膜分離装置を介して循環させる生成液循環系を備え、更に、前記培養液循環系と生成液循環系の流量バランスを保持する圧力調整手段を設けたものである点に要旨を有する細胞培養装置である。

［作用］

本発明においては、中空繊維内に培養液を加圧下に供給することによって、細胞培養空間における栄養源や溶存酸素等の枯渇を防止することを基本的な構成とするものである。この場合に中空繊維内に供給される栄養源の組成や溶存酸素量等を予め調整することによって最適な培養条件での培養液の供給が可能となる。

そして中空繊維内に供給された培養液を加圧下に循環させる培養液循環系の構成を採用することによって、前記細胞培養空間内全体に亘って均一に培養液を供給できる。又この目的をより有効に達成するには、前記培養液循環系にガス交換器好ましくは中空繊維型人工肺を設けて溶存酸素量又は溶存炭酸ガス量を制御すると共に、新鮮培養液や塩基を供給するラインを設けて上記中空繊維を透過した培養液を補い且つpHを調節するのが推奨される。

尚前記細胞培養空間内における細胞の種類は何ら限定するものではなく、付着性細胞の場合には中空繊維の外表面上に付着させる方法或はコラーゲン等の高分子ゲル状マトリックス中に閉じ込める方法（特願昭62－65363号）等が例示され、一方浮遊性細胞の倍には上記高分子マトリックス中に閉じ込める方法や液中浮遊状態で培養する方法等を挙げることができる。

細胞培養空間内の培養液は、生成した低分子不要物質及び高分子有用物質を含んだ状態で膜分離装置へ導かれる。膜分離装置へ導かれた培養液は、細胞の代謝物質である乳酸，アンモニウムイオン等の成育阻害因子となる低分子不要物質が取り除かれた後再び細胞培養空間内に循環される（生成液循環系）。尚この循環される培養液は生成液循環系内でpHや溶存酸素量が調整される。

この様に細胞培養空間内の培養液を、膜分離装置を介して循環させることによって、細胞代謝物を細胞の近傍から速やかに取り除くことができ、細胞培養空間内の環境を均一にすることができる。又このことによって、代謝物の蓄積による細胞培養空間内の部分的又は全体的なpHの急激な低下を防ぐことができる。更に、低分子不要物質を膜分離装置で選択的に分離して取り除くことによって、培養液中の成育阻害物質濃度を低下させ、且つ細胞が産生する高分子有用物質を濃縮することができる。そして高分子有用物質が濃縮された培養液を一定速度で系外へ取り出すと共に、中空繊維を介した培養液透過流量を制御して細胞培養装置内の培養液の移動が安定化されることによって、当該細胞培養装置における連続培養が可能となり、高濃度の有用物質を長期間に亘って安定し

て回収することができる様になる。
尚前記膜分離装置においては低分子物質のみが選択的に取り除かれることが望ましく、その分画分子量は少なくとも高分子有用物質が透過しない範囲としなければならず、例えば1000～200000程度好ましくは3000～30000程度とするのが適当である。一方中空繊維における分画分子量は有用物質が透過しない範囲内に設定すればよく、膜分離装置の分画分子量より大きいか又は等しくても良い。
本発明装置における中空繊維の材質は何ら限定するものではないが、例えば有機高分子、無機多孔質体又は金属多孔質体等が挙げられる。又その内径は10～1000μm、膜厚は２～500μmで良く、特に内径は50～500μm程度が好ましい。上記有機高分子材料としては、例えばセルロースアセテート，セルローストリアセテート，セルロースエステル，ポリスルホン，ポリオレフィン，ポリフルオロカーボン，ポリシロキサン等がある。
培養液循環系での循環流量は、線速度にして1000cm/分以下、特に300cm/分以下とするのが好ましく、1000cm/分を超えると中空繊維内の圧力が大きくなり過ぎ、細胞培養空間への透過量が多くなって好ましくない。
一方生成液循環系での循環流量は、線速度にして0.1～1000cm/分、特に１～300cm/分程度が好ましい。しかして0.1cm/分未満であると液の置換が達成されず、細胞の近傍における代謝物濃度が高くなり過ぎ細胞成育が阻害され、1000cm/分を超えると剪断力の影響で細胞の破壊や死滅という好ましくない現象が発生する。
前記中空繊維を透過する培養液の移動量は、例えば第１図（実施例図面）に示す様に、培養液循環系内にチャンバー７を設け、このチャンバー７内の培養液５にガス圧をかけ、この圧力を調整することによって中空繊維１内の圧力を制御し、これによって上記移動量を変化させる。そして上記チャンバー７内の培養液５の液面及び生成液循環系内に設けたチャンバー19内の培養液（生成液）の液面を夫々一定に保つ様に制御することによって、細胞培養装置内の培養液の流れが安定し、長期的な連続培養が可能となる。
以下本発明を実施例によって更に詳細に説明するが、下記実施例は本発明を限定する性質のものではなく、前・後記の趣旨に徴して各種の設計変更を加えることはいずれも本発明の技術的範囲に含まれるものである。
［実施例］
第１図は本発明の一実施例を示す概略説明図である。
本発明に係る細胞培養装置は、基本的には細胞培養器２と膜分離装置15を含んでいる。そして前記細胞培養器２は、選択透過性を有する複数の中空繊維１が容器３内に収納されて成り、中空繊維１の外周域と前記容器３との間には細胞培養空間４が形成され、該空間４内には培養されるべき細胞が充填される。
前記中空繊維１内には、送液ポンプ６によって培養液５が供給される。該培養液５はチャンバー７内に貯留されており、空気圧供給装置８により圧力が加えられることによって推進力が与えられ、培養液５の一部が細胞培養空間４内に透過してゆく。細胞培養空間４内に透過しなかった残余の培養液５は、ライン20を通ってガス交換器９に導かれ、このガス交換器９によって酸素や炭酸ガスの濃度が調整された後、新鮮培養液10と最適pH調整用塩基11（例えば0.5N－NaOH）が添加されて再びチャンバー７に戻される（培養液循環系）。又ライン20にはpHセンサー12aと溶存酸素量検知センサー13aが設けられており、ライン20内を通る培養液５のpHや溶存酸素量が測定される。
一方細胞培養空間４内の培養液５は生成した低分子物質及び高分子有用物質を含んだ状態で（以下生成液5aという）、一旦チャンバー19に貯留された後送液ポンプ14によって膜分離装置15に供給される。そして当該膜分離装置15では、前記送液ポンプ14の動圧によって分離用膜16（この実施例では中空繊維膜）を介して、生成液5a中の乳酸やアンモニウムイオン等の低分子代謝産物が分離され系外17に取り出される。尚分離用膜16の構成は図示した中空繊維膜に限らず、通常用いられている平膜状や管状の限外濾過膜や逆浸透膜であってもよい。
分離用膜16を透過しなかった生成液5aは、その後ライン21を通って再び細胞培養空間４に戻され（生成液循環系）、細胞培養空間４内が適度に撹拌され、細胞近傍の培養液５（又は生成液5a）や代謝産物等を均一に分散させるのに役立つ。そして生成液5a中のの高分子有用物質は分離膜16を透過できないので、上述した生成液循環系を経ることで濃縮されてゆく。この高分子有用物質はライン21に設けられた送液ポンプ18によって生成液5aを一定流量回収することにより、連続的に高濃度に取り出すことができ、細胞近傍の代謝産物が適当に取り除かれることと相俟って細胞培養空間４内の環境を均一に維持でき細胞を長時間に亘って高密度に培養することができる。又前記ライン21にはpHセンサー12b,溶存酸素量検知センサー13bが設けられ、ライン21内を通過する生成液のpHや溶存酸素量が測定される。
尚第１図に示した実施例は、pHセンサー12a,12b、溶存酸素量検知センサー13a,13bは培養液循環系と生成液循環系の双方に設けたけれども、どちらか一方の循環系に設ける構成であってもよい。
又この実施例においては、中空繊維１はセルロースアセテート製で分画分子量が22,000のものを、分離用膜16はセルロースアセテート製で分画分子量が5000のものを夫々用いた。
一方細胞培養装置内の培養液５や生成液5aの流れを安定させる為には、培養液循環系のチャンバー７と生成液循環系のチャンバー19の夫々に液面計22a,22bを設け、夫々のチャンバー7,19内の液面が一定となる様にチャンバー７内の圧力を空気圧供給装置８によって制御すればよ

く、こうして例えば培養液の透過量を調整することによって、前記培養液循環系と生成液循環系の流量のバランスを保持することができる。尚空気圧供給装置８をチャンバー19にも設け、チャンバー７の圧力を調整すると共にチャンバー19内の圧力を調整することによっても同様の効果が達成される。但し、この様な場合にはチャンバー７内の圧力をチャンバー19内の圧力よりも高くなる様に調整する必要がある。更に、本発明の目的を達成する為には、前記送液ポンプ6,14は、同期的に作動する脈動とすることが好ましく、又長期間無菌的状態を維持する必要性をも考慮すれば、前記送液ポンプ6,14としては密閉性に優れたベローズポンプを用いるのが最適である。

第２図は本発明の他の実施例の概略説明図であり、基本的な構成は第１図に示した構成と類似しており対応する部分には同一の参照符号を付すことにより重複説明を避ける。そしてこの実施例は、膜分離装置15から系外17に取出す生成液5aの一部をライン21に戻す様なライン23を設けたものである。即ち膜分離装置15によって分離される低分子物質の中には乳酸やアンモニウムイオン等の生育阻害物質以外にも無機塩類やアミノ酸等の様に細胞の生育に有用な栄養源も含まれており、これらの栄養源をできるだけ利用する為に第２図に示した構成が採用される。但し、この場合の返戻量は、含まれる上記生育阻害物質が細胞の生育を阻害しない程度とする必要があるのは言う迄もない。

第２図に示した構成と同様の観点から、例えば第３図に

示す様な構成も採用することができる。即ち第３図に示す構成では、膜分離装置15から系外17に取出す生成液5aの一部を培養液循環系のライン20に戻すライン24を設け、前記第２図に示した構成と同様の効果が達成される。

第４図は本発明の更に他の実施例の概略説明図である。この実施例では、系外17に生育阻害物質と共に取り出された無機塩類やアミノ酸等の栄養源を補う為に、無機塩類やアミノ酸を含んだ水溶液25を別に調製し、この水溶液25をライン26を介して前記ライン21に補給するものである。

［発明の効果］

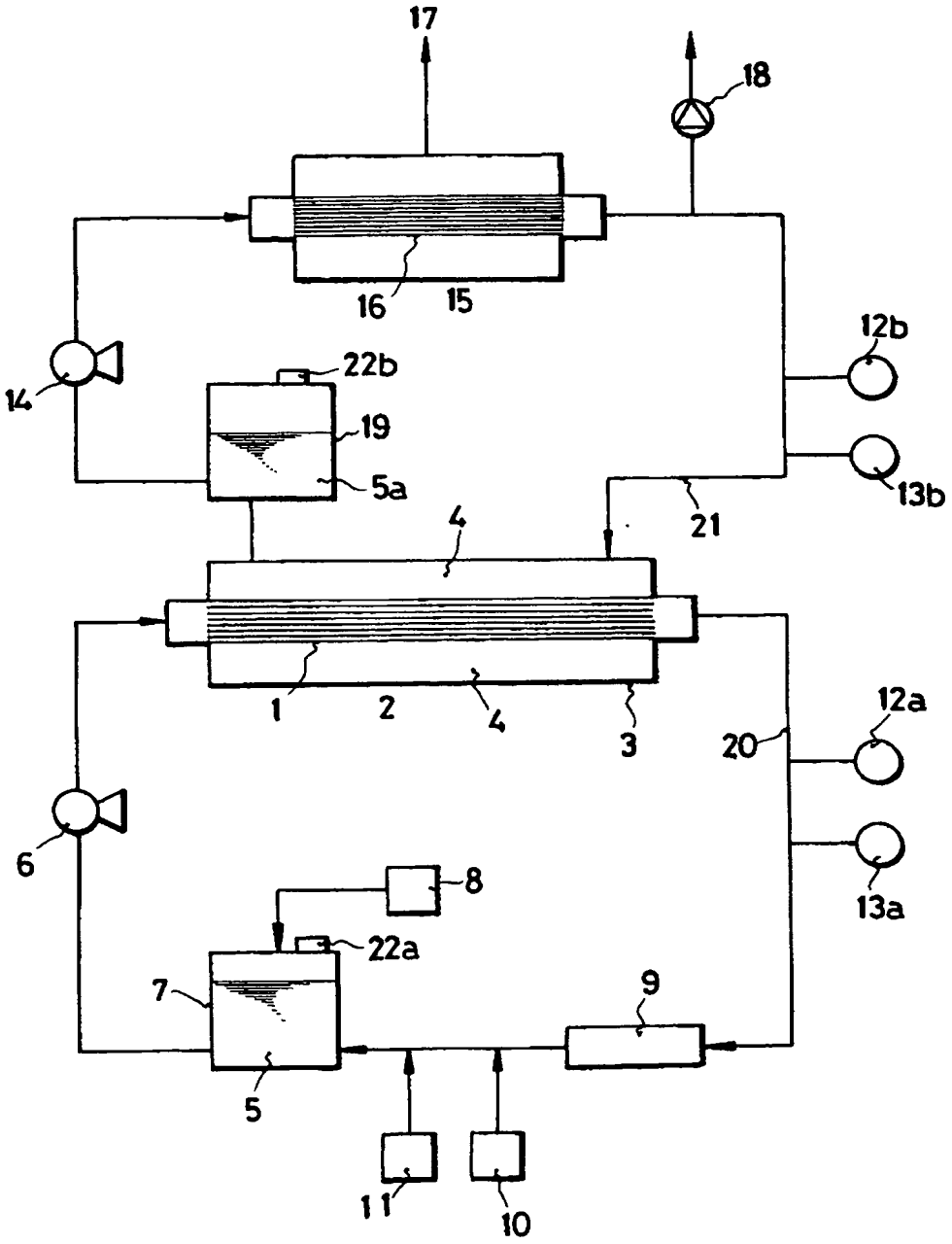
以上述べた如く本発明によれば、既述の構成を採用することによって細胞培養装置内の環境を可及的均一に維持でき、細胞の高密度な連続培養が可能となり、高濃度の有用物質を長期間に亘って得ることができる様になった。

【図面の簡単な説明】

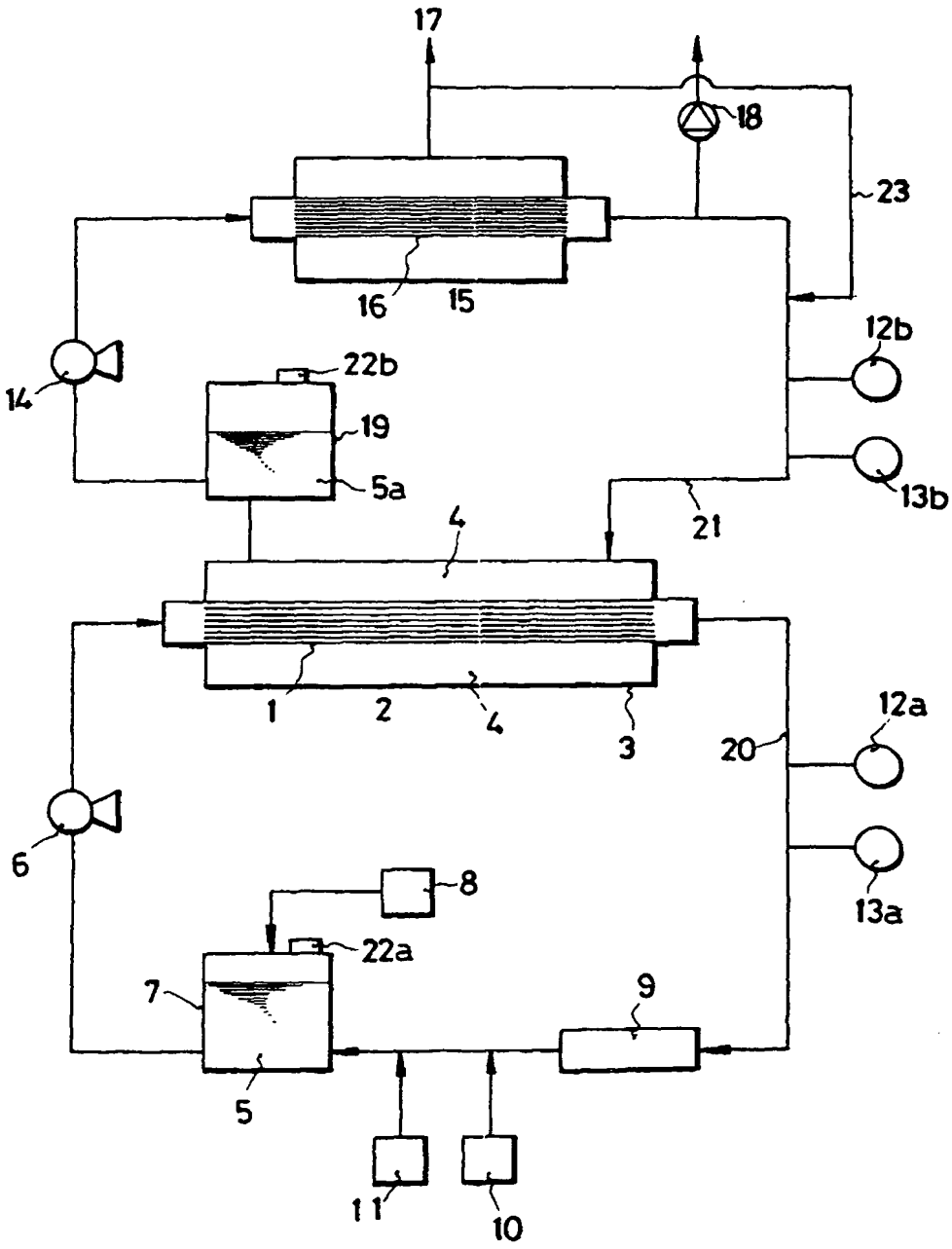
第１～第４図は本発明の各種の実施例を示す概略説明図である。

１……中空繊維、２……細胞培養器
３……容器、４……細胞培養空間
５……培養液、5a……生成液
6,14,18……送液ポンプ
7.19……チャンバー、８……空気圧供給装置
９……ガス交換器、15……膜分離装置

【第1図】


17
18
16
15
12b
14
22b
19
5a
13b
21
4
1
2
4
3
12a
20
6
8
13a
22a
7
9
5
11
10

【第２図】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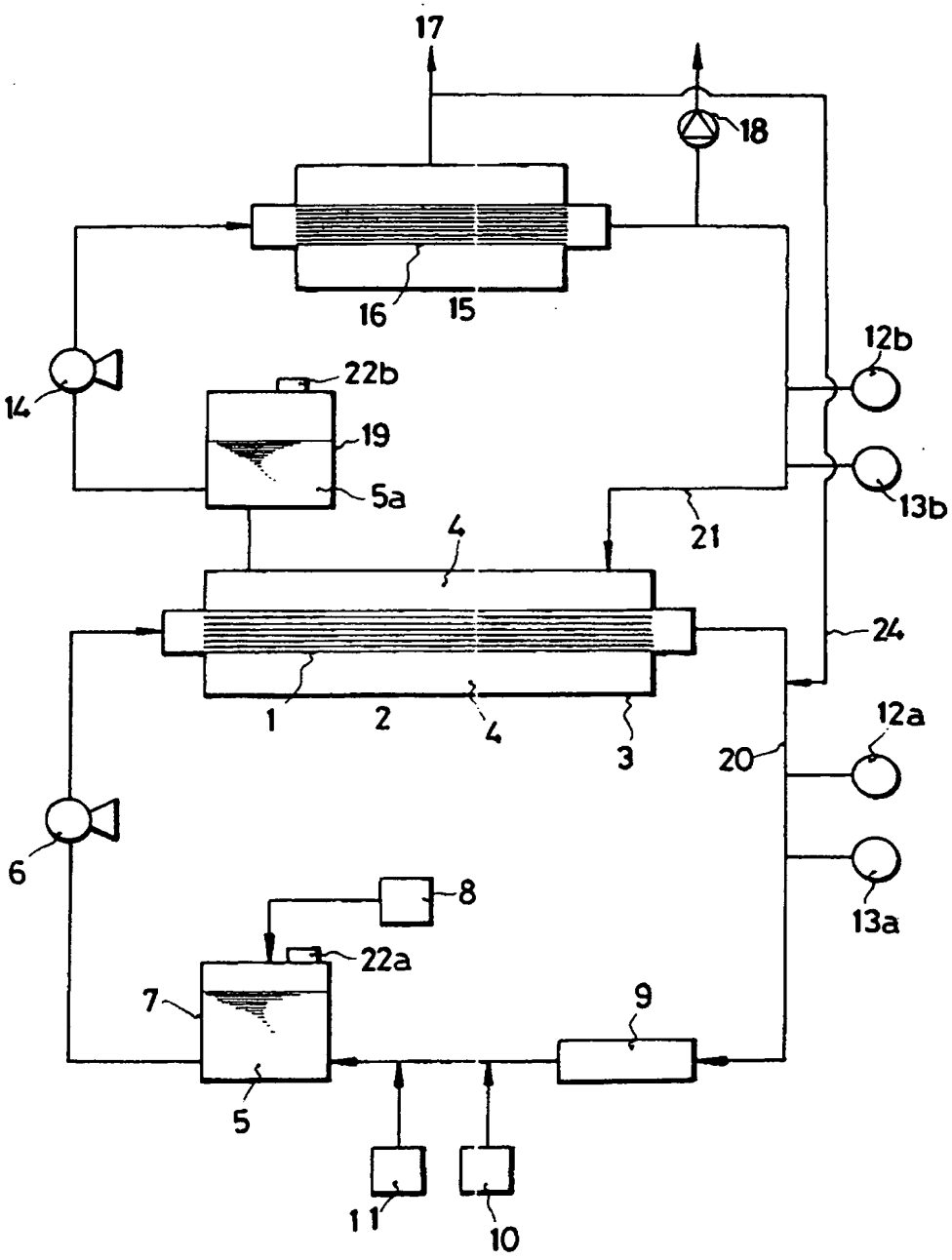
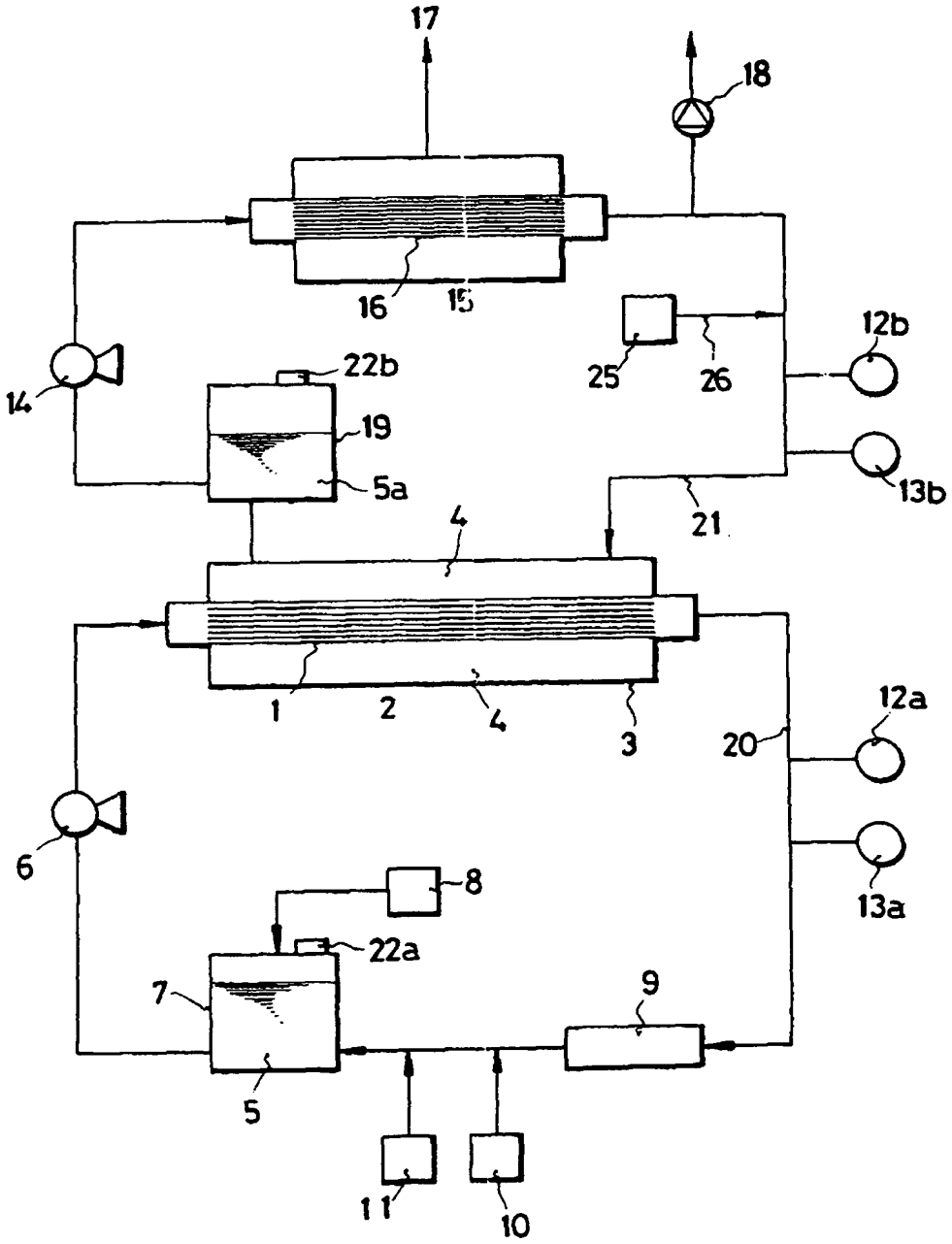
18
23
16
15
12b
14
22b
19
5a
13b
21
4
1
2
4
3
12a
20
6
8
13a
22a
7
9
5
11
10

【第3図】


17
18
16
15
12b
14
22b
19
5a
13b
21
4
24
1
2
4
3
12a
20
6
8
22a
7
9
13a
5
11
10

【第4図】


17
18
16
15
25
26
12b
14
22b
19
5a
13b
21
4
1
2
4
3
12a
20
6
8
13a
22a
7
9
5
11
10

[JP,07-097982,B]

CLAIMS DETAILED DESCRIPTION TECHNICAL FIELD PRIOR ART EFFECT OF THE INVENTION TECHNICAL PROBLEM MEANS OPERATION EXAMPLE DESCRIPTION OF DRAWINGS DRAWINGS

* NOTICES *

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3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] Cell culture equipment constituted so that a cell culture might be performed in the cell culture space which is characterized by providing the following, and which supplies culture medium under pressurization in two or more hollow fibers which have permselectivity, is made to penetrate the part to the periphery side of this hollow fiber, and is formed in the periphery region concerned. The culture medium circulatory system which circulates under pressurization the culture medium supplied in the aforementioned hollow fiber. Low-molecular matter and macromolecule useful matter which were generated in the aforementioned cell culture space.

[Translation done.]

* NOTICES *

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[Industrial Application]
It removes skillfully the undesired substance which carries out a byproduction, attains equalization of the environment in a system, this invention condensing the production useful matter while giving nutritive substance in detail to a cell efficiently about cell culture equipment, and relates to the optimal hollow-fiber type cell culture equipment for performing a continuous cell culture with high density.
[Description of the Prior Art]
High-density mass cell culture is technology indispensable when realizing production of the industrial scale of the useful physiological active substance of a monoclonal antibody, and lymphokine and others.
Various development of the method and equipment for mass cell culture has come [although the classic cell culture has been performed on laboratory level using a cultivation bottle etc. / if a cell culture can be carried out with high density and in large quantities, it is industrially advantageous, and] to be carried out from such a viewpoint.
If the cultivation method in these methods and equipment is divided roughly into a planktonic (non-adhesion) cell culture and an adhesive (wall adhesion) cell culture and is equivalent to the former planktonic cell culture with the property of the cell cultivated, it serves as the point with important on the other hand attaining [in / an adhesive cell culture / for the matter exchange with sufficient maintenance of the suspension state of a cell and sufficient efficiency] efficiently matter exchange of increase and the nutriment of the number of adherent cells per unit capacity, wastes, a gas, etc.
the above -- carry out for adopting which cultivation method -- from the place whose matter exchange is the important point The matter exchange from which the cell culture using the hollow fiber and capillary tube which have preferential segregation nature attracts attention for example, which is performed using a hollow fiber Various kinds of culture apparatus have come [while a cell obtains nutriment from blood through a blood vessel by in vivo, it is similar to discharging a metabolite, and promising ** is extremely carried out as a cell culture method of in vitro, and] to be developed. Various kinds of improvement, such as performing positively the gas exchange of the method of passing a liquid medium (culture medium) in a hollow fiber, making a cell adhere to a hollow-fiber outside surface, and proliferating especially, and the cell which passes the gas containing oxygen in a hollow fiber, and exists in the liquid medium in a hollow-fiber outside surface, is advanced. as cell culture technology using such a hollow fiber, technology, such as JP,49-41579,A, 50-36684, 52-125688, 59-175877, 61-25477, the official announcement patent No. 500356 [Showa 62 to], and 62-500357, is indicated by this
[Problem(s) to be Solved by the Invention]
As a limiting factor to the high density cultivation of a cell, accumulation of the growth inhibitor (for example, metabolites, such as a lactic acid and an ammonium ion) within a system, an exhaustion of indispensable nutrients (for example, a glucose, an essential amino acid, a vitamin, etc.), an exhaustion of a dissolved acid quantum, the rapid fall of pH, etc. are mentioned further. Although developed for the purpose of eliminating such a limiting factor completely with

conventional hollow-fiber type cell culture equipment, it was the situation which is hard to be called technology which established the high-density cell culture. The actual condition was a perfect uniform state's not becoming, but it becoming inadequate partially growing [of a cell] the environment especially in a culture apparatus, and not becoming so high that the cell density as the whole culture apparatus being expected as a result.
The place which this invention is made in order to solve such a technical technical problem, and is made into the purpose makes environment in cell culture equipment as much as possible uniform, and is to offer the hollow-fiber type cell culture equipment which can perform a cell culture with high density by this.
[Means for Solving the Problem]
With this invention which could attain the above-mentioned purpose, supply culture medium under pressurization in two or more hollow fibers which have permselectivity, and the part is made to penetrate to the periphery side of this hollow fiber. In the cell culture equipment constituted so that a cell culture might be performed in the cell culture space formed in the periphery region concerned The culture medium circulatory system which circulates under pressurization the culture medium supplied in the aforementioned hollow fiber, The culture medium containing the low-molecular matter and macromolecule useful matter which were generated in the aforementioned cell culture space It is cell culture equipment which is equipped with the generation liquid circulatory system circulated through the membrane-separation equipment for separating the aforementioned low-molecular matter, and has a summary further at the point of establishing a pressure regulation means to hold the flow rate balance of the aforementioned culture medium circulatory system and the generation liquid circulatory system.
[Function]
In this invention, it considers preventing an exhaustion of the nutrient in cell culture space, dissolved oxygen, etc. as fundamental composition by supplying culture medium under pressurization in a hollow fiber. In this case, supply of the culture medium in the optimal culture condition is attained by adjusting beforehand composition of a nutrient, a dissolved acid quantum, etc. which are supplied in a hollow fiber.
And by adopting the composition of the culture medium circulatory system which circulates under pressurization the culture medium supplied in the hollow fiber, it continues in [aforementioned / whole] cell culture space, and culture medium can be supplied uniformly. moreover -- for attaining this purpose more effectively -- the aforementioned culture medium circulatory system -- a gas exchange machine -- while preparing a hollow-fiber type artificial lung preferably and controlling a dissolved acid quantum or the amount of dissolved carbon dioxide gas, compensating the culture medium which prepared the line which supplies fresh culture medium and a base, and penetrated the above-mentioned hollow fiber, and adjusting pH is recommended
In addition, it does not limit at all, the method (Japanese Patent Application No. No. 65363 [62 to]) of shutting up into macromolecule gel matrices, such as the method of making it adhere on the outside surface of a hollow fiber in the case of an adhesive cell or a collagen, etc. is illustrated, and, on the other hand, the kind of cell in the aforementioned cell culture space can mention the method of shutting up into the above-mentioned macromolecule matrix, the method of cultivating in the state of suspension among liquid, etc. the twice of a planktonic cell.
The culture medium in cell culture space is led to membrane-separation equipment, where the low-molecular undesired substance and the macromolecule useful matter which were generated are included. After the low-molecular undesired substance used as growth inhibitor which is the metabolites of a cell, such as a lactic acid and an ammonium ion, is removed, it circulates through the culture medium led to membrane-separation equipment in cell culture space again (generation liquid circulatory system). In addition, as for this culture medium through which it circulates, pH and a dissolved acid quantum are adjusted within the generation liquid circulatory system.
Thus, by circulating the culture medium in cell culture space through membrane-separation equipment, a cell metabolite can be promptly removed from near the cell, and environment in cell culture space can be made uniform. Moreover, this can protect the rapid fall of partial or overall

pH in the cell culture space by accumulation of a metabolite. Furthermore, the macromolecule useful matter which the growth inhibitor concentration in culture medium is reduced, and a cell produces can be condensed by dissociating alternatively and removing low-molecular undesired substance with membrane-separation equipment. And while taking out the culture medium by which the macromolecule useful matter was condensed out of a system by constant speed, by controlling the culture medium transparency flow rate through the hollow fiber, and stabilizing movement of the culture medium in cell culture equipment, the continuous culture in the cell culture equipment concerned becomes possible, it continues at a long period of time, is stabilized, and high-concentration useful matter can be collected.
In addition, it is desirable to remove only the low-molecular matter alternatively in the aforementioned membrane-separation equipment, and it is appropriate for the cut off molecular weight that it must consider as the range which the macromolecule useful matter does not penetrate at least, for example, takes preferably about for 3000 to 30000 1000 to about 200000. or [that it is / that what is necessary is just to, set up the cut off molecular weight in a hollow fiber within limits which the useful matter does not penetrate on the other hand / larger than the cut off molecular weight of membrane-separation equipment] -- or it may be equal
Although the quality of the material of the hollow fiber in this invention equipment is not limited at all, an organic macromolecule, an inorganic porosity object, or a metal porosity object is mentioned, for example. Moreover, 10-1000 micrometers and the thickness of the bore are good at 2-500 micrometers, and especially a bore has desirable about 50-500 micrometers. As the above-mentioned organic polymeric materials, there are a cellulose acetate, a cellulose triacetate, a cellulose ester, a polysulfone, a polyolefine, the poly fluorocarbon, a polysiloxane, etc., for example.
It is made linear velocity, and by 1000cm/, it is desirable to consider as the following by 300cm/especially hereafter, and it is not [if a part for 1000cm/is exceeded, the pressure in a hollow fiber will become large too much, the amount of transparency of the amount / of circulating flows / in the culture medium circulatory system to cell culture space increases, and] desirable.
On the other hand, the amount of circulating flows in the generation liquid circulatory system is made into linear velocity, and its minute is especially desirable in about 1-300cm /by 0.1-1000cm/. A deer is carried out, the substitution of liquid is not attained as it is the following by 0.1cm/, but metabolite concentration [near the cell] becomes high too much, cell growth is checked, and if a part for 1000cm/is exceeded, the phenomenon of destruction and extinction of a cell which is not desirable will occur under the influence of shearing force.
As shown for example, in the 1st view (example drawing), the movement magnitude of the culture medium which penetrates the aforementioned hollow fiber forms a chamber 7 in the culture medium circulatory system, applies gas ** to the culture medium 5 in this chamber 7, by adjusting this pressure, controls the pressure in a hollow fiber 1, and changes the above-mentioned movement magnitude by this. And by controlling to keep constant the oil level of the culture medium in the chamber 19 prepared in the oil level of the culture medium 5 in the above-mentioned chamber 7, and the generation liquid circulatory system (generation liquid), respectively, the flow of the culture medium in cell culture equipment is stabilized, and a long-term continuous culture becomes possible.
Although an example explains this invention still in detail below, the following example is not the thing of the property which limits this invention, and each thing for which before and the after-mentioned meaning are marked and various kinds of design changes are added is included in the technical range of this invention.
[Example]
A view 1 is outline explanatory drawing showing one example of this invention.
The cell culture equipment concerning this invention contains the cell culture machine 2 and membrane-separation equipment 15 fundamentally. And two or more hollow fibers 1 in which the aforementioned cell culture machine 2 has permselectivity are contained in a container 3, it changes, the cell culture space 4 is formed between the periphery region of a hollow fiber 1, and the aforementioned container 3, and it fills up with the cell which should be cultivated in this

space 4.
In the aforementioned hollow fiber 1, culture medium 5 is supplied with the liquid-sending pump 6. This culture medium 5 is stored in the chamber 7, by applying a pressure by the pneumatic pressure feeder 8, driving force is given and a part of culture medium 5 penetrates it in the cell culture space 4. The fresh culture medium 10 and the base 11 (for example, 0.5 N-NaOH) for optimum-pH adjustment are added, and the culture medium 5 of the remainder which was not penetrated in the cell culture space 4 is again returned to a chamber 7, after it is led to the gas exchange machine 9 through a line 20 and the concentration of oxygen or carbon dioxide gas is adjusted by this gas exchange machine 9 (culture medium circulatory system). Moreover, pH sensor 12a and dissolved acid quantum detection sensor 13a are prepared in the line 20, and pH and the dissolved acid quantum passing through the inside of a line 20 of culture medium 5 are measured.
On the other hand, the culture medium 5 in the cell culture space 4 is supplied to membrane-separation equipment 15 with (it is called generation liquid 5a below) and the post-liquid-sending pump 14 once stored by the chamber 19, where the low-molecular matter and macromolecule useful matter which were generated are included. And with the membrane-separation equipment 15 concerned, through the film 16 (this example hollow-fiber film) for separation, low-molecular metabolites, such as a lactic acid in generation liquid 5a and an ammonium ion, are separated, and it is taken out by the dynamic pressure of the aforementioned liquid-sending pump 14 17 outside a system. In addition, the composition of the film 16 for separation may be not only the illustrated hollow-fiber film but the shape of a flat film usually used and tubular extra ******, and a reverse osmosis membrane.
It is again returned to the cell culture space 4 through a line 21 after that (generation liquid circulatory system), the inside of the cell culture space 4 is agitated moderately, and generation liquid 5a which did not penetrate the film 16 for separation is useful to distributing uniformly culture medium 5 (or generation liquid 5a), a metabolite, etc. near the cell. And since the macromolecule useful matter of ** in generation liquid 5a cannot penetrate a demarcation membrane 16, it is condensed by passing through the generation liquid circulatory system mentioned above. By carrying out constant-flow recovery of the generation liquid 5a with the liquid-sending pump 18 formed in the line 21, this macromolecule useful matter can be continuously taken out to high concentration, the environment in the cell culture space 4 can be conjointly maintained uniformly with the metabolite near the cell being removed suitably, and can cover a long time and can cultivate a cell with high density. Moreover, pH sensor 12b and dissolved acid quantum detection sensor 13b are prepared in the aforementioned line 21, and pH and the dissolved acid quantum of generation liquid which pass through the inside of a line 21 are measured.
The example shown in the **** 1 view may be composition prepared in one of the circulatory system although the pH sensors 12a and 12b and the dissolved acid quantum detection sensors 13a and 13b are prepared for the both sides of the culture medium circulatory system and the generation liquid circulatory system and can be cooked.
Moreover, in this example, the hollow fiber 1 was a product made from a cellulose acetate, the cut off molecular weight used the thing of 22,000, and, as for the film 16 for separation, the cut off molecular weight used the thing of 5000 by the product made from a cellulose acetate, respectively.
On the other hand, in order to stabilize the culture medium 5 in cell culture equipment, and the flow of generation liquid 5a Level gages 22a and 22b are formed in each of the chamber 7 of the culture medium circulatory system, and the chamber 19 of the generation liquid circulatory system. The balance of the flow rate of the aforementioned culture medium circulatory system and the generation liquid circulatory system can be held by adjusting the amount of transparency of culture medium in this way, for example that what is necessary is just to control the pressure in a chamber 7 by the pneumatic pressure feeder 8 so that the oil level in each chamber 7 and 19 becomes fixed. In addition, the pneumatic pressure feeder 8 is formed also in a chamber 19, and while adjusting the pressure of a chamber 7, the same effect is attained also by adjusting the pressure in a chamber 19. However, when such, it is necessary to adjust the pressure in a

chamber 7 so that it may become higher than the pressure in a chamber 19. Furthermore, in order to attain the purpose of this invention, as for the aforementioned liquid-sending pumps 6 and 14, considering as the pulsation which operates in synchronization is desirable, and if the need of maintaining a sterile state for a long period of time is also taken into consideration, it is optimal to use the bellows pump which was excellent in sealing nature as the aforementioned liquid-sending pumps 6 and 14.

A view 2 is outline explanatory drawing of other examples of this invention, and fundamental composition avoids duplication explanation by giving the same reference mark to the portion which is similar with the composition shown in the view 1, and corresponds. And this example forms the line 23 which returns a part of generation liquid 5a taken out from membrane-separation equipment 15 to 17 outside a system to a line 21. That is, in the low-molecular matter separated by membrane-separation equipment 15, the useful nutrient is also contained in growth of a cell like mineral or amino acid besides growth-inhibition matter, such as a lactic acid and an ammonium ion, and in order to make the most of these nutrients, the composition shown in the view 2 is adopted. However, it is not that ****** in this case needs to consider as the grade from which the above-mentioned growth-inhibition matter contained does not prevent growth of a cell, either, until it says.

From the same viewpoint as the composition shown in the view 2, composition as shown, for example in a view 3 is also employable. That is, with the composition shown in the 3rd view , the line 24 which returns a part of generation liquid 5a taken out from membrane-separation equipment 15 to 17 outside a system to the line 20 of the culture medium circulatory system is formed, and the same effect as the composition shown in the view 2 of the above is attained.

A view 4 is outline explanatory drawing of the example of further others of this invention. In this example, in order to compensate nutrients taken out with the growth-inhibition matter by 17 outside a system, such as mineral and amino acid, the solution 25 containing mineral or amino acid is prepared independently, and this solution 25 is supplied to the aforementioned line 21 through a line 26.

[Effect of the Invention]

According to this invention, as stated above, by adopting composition as stated above, the environment in cell culture equipment could be maintained as much as possible uniformly, the continuous culture with a high-density cell became possible, it continues at a long period of time, and the high-concentration useful matter can be obtained now.

[Translation done.]

* NOTICES *

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
A view 1st-4 is outline explanatory drawing showing various kinds of examples of this invention.
1 .... A hollow fiber, 2 .. Cell culture machine
3 .... A container, 4 .. Cell culture space
5 .... Culture medium, 5a .. Generation liquid
6, 14, 18 .... Liquid-sending pump
7.19 .... A chamber, 8 .. Pneumatic pressure feeder
9 .... A gas exchange machine, 15 .. Membrane-separation equipment

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[Translation done.]